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In the Specification:

On pages 1-2, please delete the paragraph at lines 24-36 and 1-6 and replace it with the following paragraph (Please note that the line numbering on page 1 is incorrect. According to the numbering currently on the page, the line numbers on page 1 are 12-24):

The development of effective cancer therapies has been a major focus of biomedical research. Surgical procedures have been developed and used to treat patients whose tumors are confined to particular anatomical sites. However, at present, only about 25% of patients have tumors that are truly confined and amenable to surgical treatment alone (Slapak et al. in Harrison's Principles of Internal Medicine (Isselbacher et al., eds.) McGraw-Hill, Inc., NY (1994) pp. 1826-1850). Radiation therapy, like surgery, is a local modality whose usefulness in the treatment of cancer depends to a large extent on the inherent radiosensitivity of the tumor and its adjacent normal tissues. However, radiation therapy is associated with both acute toxicity and long term sequelae. Furthermore, radiation therapy is known to be mutagenic, carcinogenic, and teratogenic (Slapak et al., ibid.).

On pages 3-4, please delete the paragraph at lines 14-32 and 1-9 and replace it with the following paragraph:

One such gene encodes the RI_α subunit of cyclic AMP (cAMP)-dependent protein kinase A (PKA) (Krebs (1972) *Curr. Topics Cell. Regul.* **5**:99-133). Protein kinase is bound by cAMP, which is thought to have a role in the control of cell proliferation and differentiation (see, e.g., Cho-Chung (1980) *J. Cyclic Nucleotide Res.* **6**:163-167). There are two types of PKA, type I (PKA-I) and type II (PKA-II), both of which share a common C subunit but each containing distinct R subunits, RI and RII, respectively (Beebe et al. in *The Enzymes: Control by Phosphorylation*, 17(A):43-111 (Academic, New York, 1986). The R subunit isoforms differ in tissue distribution (Øyen et al. (1988) *FEBS Lett.* **229**:391-394; Clegg et al. (1988) *Proc. Natl. Acad. Sci. (USA)* **85**:3703-3707) and in biochemical properties (Beebe et al. in *The Enzymes: Control by Phosphorylation*, 17(A):43-111 (Academic Press, NY, 1986); Cadd et al. (1990) *J. Biol. Chem.* **265**:19502-19506). The two general isoforms of the R subunit also differ in their subcellular localization: RI is found throughout the cytoplasm; whereas RII localizes to nuclei, nucleoli, Golgi apparatus and the microtubule-organizing center (see, e.g., Lohmann in *Advances in Cyclic Nucleotide and Protein Phosphorylation Research*, 18:63-117 (Raven, New York, 1984; and Nigg et al. (1985) *Cell* **41**:1039-1051).

On page 5, please delete the paragraph at lines 7-18 and replace it with the following paragraph:

Antisense oligonucleotides directed to the RI_α gene have been prepared. U.S. Patent No. 5,271,941 describes phosphodiester-linked antisense oligonucleotides complementary to a region of the first 100 N-terminal amino acids of RI_α which inhibit the expression of RIα in leukemia cells *in vitro*. In addition, antisense phosphorothioate oligodeoxynucleotides corresponding to the N-terminal 8-13 codons of the RI_α gene was found to reduce *in vivo* tumor growth in nude mice (Nesterova et al. (1995) *Nature Med.* **1**:528-533).

On pages 10-11, please delete the paragraph at lines 22-34 and 1-28 and replace it with the following paragraph:

In another preferred embodiment according to this aspect of the invention, the oligonucleotide is an inverted hybrid oligonucleotide comprising a region of at least four ribonucleotides flanked by 3′ and 5′ deoxyribonucleotide regions of at least two deoxyribonucleotides. The structure of this oligonucleotide is "inverted" relative to traditional hybrid oligonucleotides. In some embodiments, the 2′-O-substituted RNA region has from about four to about ten 2′-O-substituted nucleosides joined to each other by 5′ to 3′ internucleoside linkages, and most preferably from about four to about six such 2′-O-substituted nucleosides. In some embodiments, the oligonucleotides of the invention have a ribonucleotide region that comprises at least five

contiguous ribonucleotides. In one particularly preferred embodiment, the overall size of the inverted hybrid oligonucleotide is 18. In preferred embodiments, the 2'-O-substituted ribonucleosides are linked to each other through a 5' to 3' phosphorothioate, phosphorodithioate, phosphotriester, or phosphodiester linkages. The phosphorothioate 3' or 5' flanking region (or regions) has from about four to about 18 nucleosides joined to each other by 5' to 3' phosphorothioate linkages, and preferably from about 5 to about 18 such phosphorothioate-linked nucleosides. In preferred embodiments, the phosphorothioate regions will have at least 5 phosphorothioate-linked nucleosides. One specific embodiment is an oligonucleotide having substantially the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:6. In preferred embodiments of this aspect of the invention, the ribonucleotide region comprises 2'-O-substituted ribonucleotides, such as 2'-O-alkyl substituted ribonucleotides. One particularly preferred embodiment is an inverted hybrid oligonucleotide whose ribonucleotide region comprises at least one 2'-O-methyl ribonucleotide.

On pages 11-12, please delete the paragraph at lines 30-34 and 1-12 and replace it with the following paragraph:

In some embodiments, all of the nucleotides in the inverted hybrid oligonucleotide are linked by phosphorothioate internucleotide linkages. In particular embodiments, the deoxyribonucleotide flanking region or regions has from about four to about 18 nucleosides joined to each other by 5' to 3' phosphorothioate linkages, and preferably from about 5 to about 18 such phosphorothioate-linked nucleosides. In some embodiments, the deoxyribonucleotide 3' and 5' flanking regions of the inverted hybrid oligonucleotides of the invention have about 5 phosphorothioate-linked nucleosides. The phosphorothioate linkages may be mixed R_p and S_p enantiomers, or they may be stereoregular or substantially stereoregular in either R_p or S_p form (see Iyer et al. (1995) *Tetrahedron Asymmetry* **6**:1051-1054).

On page 17, please delete the paragraph at lines 14-28 and replace it with the following paragraph:

Those skilled in the art will recognize that the elements of these preferred embodiments can be combined and the inventor does contemplate such combination. For example, 2'-O-substituted ribonucleotide regions may well include from one to all nonionic internucleoside linkages. Alternatively, nonionic regions may have from one to all 2'-O-substituted ribonucleotides. Moreover, oligonucleotides according to the invention may contain combinations of one or more 2'-O-substituted ribonucleotide region and one or more nonionic region, either or both being flanked by phosphorothioate regions. (See *Nucleosides & Nucleotides* **14:**1031-1035 (1995) for relevant synthetic techniques.)

On page 20, please delete the paragraph at lines 27-29 and replace it with the following paragraph:

FIG. 18 is a tabular representation of histochemical analysis of GEO tumors following treatment with taxol and/or different oral MBOs.

On page 21, please delete the paragraph at lines 14-28 and replace it with the following paragraph:

Such synthetic hybrid, inverted hybrid, and inverted chimeric oligonucleotides of the invention have a nucleotide sequence complementary to a genomic region or an RNA molecule transcribed therefrom encoding the RI_α subunit of PKA. These oligonucleotides are about 15 to about 30 nucleotides in length, preferably about 15 to 25 nucleotides in length, but most preferably, are about 18 nucleotides long. The sequence of this gene is known. Thus, an oligonucleotide of the invention can have any nucleotide sequence complementary to any region of the gene. Three non-limiting examples of an 18mer of the invention has the sequence set forth below in TABLE 1 as SEQ ID NOS:1, 4, and 6.

On page 25, please delete the paragraph at lines 4-14 and replace it with the following paragraph:

The invention also provides therapeutic compositions suitable for treating undesirable, uncontrolled cell proliferation or cancer comprising at least one oligonucleotide in accordance with the invention, capable of specifically down-regulating expression of the RI_α gene, and a pharmaceutically acceptable carrier or diluent. It is preferred that an oligonucleotide used in the therapeutic composition of the invention be complementary to at least a portion of the RI_α genomic region, gene, or RNA transcript thereof.

On page 25, please delete the paragraph at lines 16-29 and replace it with the following paragraph:

As used herein, a "pharmaceutically or physiologically acceptable carrier" includes any and all solvents (including but not limited to lactose), dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions of the invention is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

On pages 25-26 please delete the paragraph at lines 31-34 and 1-6 and replace it with the following paragraph:

Several preferred therapeutic compositions of the invention suitable for inhibiting cell proliferation *in vitro* or *in vivo* or for treating cancer in humans in accordance with the methods of the invention comprise about 25 to 75 mg of a lyophilized oligonucleotide(s) having SEQ ID NOS:1, 4, and/or 6 and 20-75 mg lactose, USP, which is reconstituted with sterile normal saline to the therapeutically effective dosages described herein.

On page 26, please delete the paragraph at lines 8-15 and replace it with the following paragraph:

In another aspect, the invention provides pharmaceutical compositions comprising a modified oligonucleotide of the invention in combination with an antibody that binds to epidermal growth factor receptor (EGFR) or a cytotoxic agent. Preferred cytotoxic agents include, without limitation, taxanes, platinum-derived agents, and topoisomerase II-selective drugs.

On page 28, please delete the paragraph at lines 5-14 and replace it with the following paragraph:

In preferred embodiments according to this aspect of the invention, the first agent is a synthetic modified oligonucletotide having a nucleotide sequence consisting essentially of the nucleotide sequence set forth in SEQ ID NO:4. Preferably, the oligonucleotide is administered at a dose of up to 540 mg/m^2/dose by intravenous infusion (2 hours to 21 days in duration or up to 1,050 mg/m^2/day by oral or rectal administration.

On pages 28-29, please delete the paragraph at lines 31-34 and 1-6 and replace it with the following paragraph:

A "therapeutically effective manner" refers to a route, duration, and frequency of administration of the pharmaceutical formulation which ultimately results in meaningful patient benefit, as described above. In some embodiments of the invention, the pharmaceutical formulation is administered via injection, sublingually, rectally, intradermally, orally, or enterally in bolus, continuous, intermittent, or continuous, followed by intermittent, regimens.

On pages 29-30, please delete the paragraph at lines 8-34 and 1-10 and replace it with the following paragraph:

The therapeutically effective amount of synthetic oligonucleotide in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of synthetic oligonucleotide with which to treat each individual patient. Initially, the attending physician will administer low doses of the synthetic oligonucleotide and observe the patient's response. Larger doses of synthetic oligonucleotide may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the dosages of the pharmaceutical compositions administered in the method of the present invention should contain about 0.1 to 5.0 mg/kg

body weight per day, and preferably 0.1 to 2.0 mg/kg body weight per day. When administered systemically, the therapeutic composition is preferably administered at a sufficient dosage to attain a blood level of oligonucleotide from about 0.01 μM to about 10 μM. Preferably, the concentration of oligonucleotide at the site of aberrant gene expression should be from about 0.01 μM to about 10 μM, and most preferably from about 0.05 μM to about 5 μM. However, for localized administration, much lower concentrations than this may be effective, and much higher concentrations may be tolerated. It may be desirable to administer simultaneously or sequentially a therapeutically effective amount of one or more of the therapeutic compositions of the invention to an individual as a single treatment episode.

On page 30 please delete the paragraph at lines 12-22 and replace it with the following paragraph:

Administration of pharmaceutical compositions in accordance with the invention or to practice the method of the present invention can be carried out in a variety of conventional ways, such as by oral ingestion, enteral, rectal, or transdermal administration, inhalation, sublingual administration, or cutaneous, subcutaneous, intramuscular, intraocular, intraperitoneal, or intravenous injection, or any other route of administration known in the art for administrating therapeutic agents.

On page 32, please delete the paragraph at lines 10-28 and replace it with the following paragraph:

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile. It must be stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents. Prolonged absorption of the injectable therapeutic agents can be brought about by the use of compositions of agents delaying absorption. Sterile injectable solutions are prepared by incorporating the oligonucleotide in the required amount in the appropriate solvent, followed by filtered sterilization.

On page 34, please delete the paragraph at lines 20-33 and replace it with the following paragraph:

At least one therapeutic composition of the invention may be administered in accordance with the method of the invention either alone or in combination with other known therapies for cancer such as cisplatin, carboplatin, paclitaxel, tamoxifen, taxol, interferon α and doxorubicin. When co-administered with one or more other therapies, the compositions of the invention may be administered either simultaneously with the other treatment(s), or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering the compositions of the invention in combination with the other therapy.

On pages 36-37, please delete the paragraph at lines 29-34 and 1-21 and replace it with the following paragraph:

The cell line utilized was the CEM-SS cell line (Southern Research Institute-Frederick Research Center, Frederick, MD). These cells are highly susceptible to infection with HIV, rapidly form multinucleated syncytia, and are eventually killed by HIV. The cells were maintained (2-7 x 10^5 cells per ml) in RPMI 1640 tissue culture medium supplemented with 10% fetal bovine serum, glutamine, and antibiotics, and were passaged twice weekly at 1:20 dilution. Passage number was logged each week. Cells were discarded after twenty weeks of passage and fresh CEM-SS cells thawed and utilized in the assay. Stocks of CEM-SS cells were frozen in liquid nitrogen in 1 ml NUNC vials in 90% fetal calf serum and 10% dimethyl sulfoxide (DMSO). Following thawing, CEM-SS cells were routinely ready to be utilized in the primary screen assay after two weeks in culture. Prior to replacing a late passage cell line, the new CEM-SS cells were tested in the screening assay protocol utilizing the current stock of infectious virus and AZT. If the infectivity of the virus was significantly different on the new cells or if AZT appeared less active than expected the new cells were not entered into the screening program. Mycoplasma testing was routinely performed on all cell lines.

On page 39, please delete the paragraph at lines 25-30 and replace it with the following paragraph:

Titer determinations included reverse transcriptase activity assay (see methods below), endpoint titration or plaque assay (CEM-SS) quantification of infectious particles (see methods below), and quantification of cell killing kinetics.

On pages 43-44, please delete the paragraph at lines 18-34 and 1-2 and replace it with the following paragraph:

ELISA kits were purchased from Coulter. The assay is performed according to the manufacturer's recommendations. Prior to ELISA analysis we routinely performed the reverse transcriptase activity assays (described above) and used the values for incorporated radioactivity in the RT activity assay to determine the dilution of our samples required for the ELISA. We have constructed standard curves so that the dilutions of virus to be used in the p24 ELISA can be accurately determined from the RT activity assay. Control curves are generated in each assay to accurately quantify the amount of capsid protein in each sample. Data was obtained by spectrophotometric analysis at 450 nm using a Molecular Devices Vmax plate reader. P24 concentrations were calculated from the optical density values by use of the Molecular Devices software package Soft Max.

On page 44 please delete the paragraph at lines 6-25 and replace it with the following paragraph:

Infectious virus particles were quantified utilizing the CEM-SS plaque assay as described by Nara, P.L. and Fischinger, P.J. (1988) Quantitative infectivity assay for HIV-1 and HIV-2 *Nature* 332:469-470). Flat bottom 96-well microtiter plates (Costar) were coated with 50 µl of poly-L-lysine (Sigma) at 50 µg/ml for 2 hours at 37°C. The wells were then washed with PBS and 2.5×10^5 CEM-SS cells were placed in the microtiter well where they became fixed to the bottom of the plate. Enough cells were added to form a monolayer of CEM-SS cells in each well. Virus containing supernatant was added from each well of the XTT plate, including virus and cell controls and each serial dilution of the test substance. The number of syncytia were quantified in the flat-bottom 96-well microtiter plate with an Olympus CK2 inverted microscope at 4 days following infection. Each syncytium resulted from a single infectious HIV virion.

On pages 48-50, please delete the paragraph at lines 27-34, 1-34, and 1-11 and replace it with the following paragraph:

To determine the relative effect of inverted hybrid or inverted chimeric structure on oligonucleotide-mediated depletion of complement, the following experiments were performed. Venous blood was collected from healthy adult human volunteers. Serum was prepared for hemolytic complement assay by collecting blood into vacutainers (Becton Dickinson #6430 Franklin

Lakes, NJ) without commercial additives. Blood was allowed to clot at room temperature for 30 minutes, chilled on ice for 15 minutes, then centrifuged at 4°C to separate serum. Harvested serum was kept on ice for same day assay or, alternatively, stored at -70°C. Buffer, or an oligonucleotide sample was then incubated with the serum. The oligonucleotides tested were 25mer oligonucleotide phosphodiesters or phosphorothioates, 25mer hybrid oligonucleotides, 25mer inverted hybrid oligonucleotides, 25mer chimeric oligonucleotides, and 25mer inverted chimeric oligonucleotides. Representative hybrid oligonucleotides were composed of seven to 13 2'-O-methyl ribonucleotides flanked by two regions of six to nine deoxyribonucleotides each. Representative 25mer inverted hybrid oligonucleotides were composed of 17 deoxyribonucleotides flanked by two regions of four ribonucleotides each. Representative 25mer chimeric oligonucleotides were composed of six methylphosphonate deoxyribonucleotides and 19 phosphorothioate deoxyribonucleotides. Representative inverted chimeric oligonucleotides were composed of from 16 to 17 phosphorothioate deoxyribonucleotides flanked by regions of from two to seven methylphosphonate deoxyribonucleotides, or from six to eight methylphosphonate deoxyribonucleotides flanked by nine to ten phosphorothioate deoxyribonucleotides, or two phosphorothioate regions ranging from two to 12 oligonucleotides, flanked by three phosphorothioate regions ranging in size from two to six nucleotides in length. A standard CH50 assay (See Kabat and Mayer (eds), *Experimental*

Immunochemistry, 2d Ed., Springfield, IL, CC Thomas, p. 125) for complement-mediated lysis of sheep red blood cells (Colorado Serum Co.) sensitized with anti-sheep red blood cell antibody (hemolysin, Diamedix, Miami, FL) was performed, using duplicate determinations of at least five dilutions of each test serum, then hemoglobin release into cell-free supernates was measured spectrophotometrically at 541 nm.

On page 70, please delete the paragraph at lines 3-11 and replace it with the following paragraph:

Two different 18-mer MBOs complementary to the RIα subunit of PKAI sequence, HYB 165 and its control oligomer HYB 508, differing only in 4 nucloetide bases, were tested to study their effect on soft agar growth of 1A9 human ovarian cancer cells. While HYB 165 determined a dose-dependent inhibition of colony formation at does ranging between 0.1 and 2.5 μM in all cell lines, the HYB 508 control sequence showed a modest or no growth inhibitory effect. HYB 165 determined an inhibition of 1A9PTX22 cell growth of approximately 5% at a dose of 0.1 μM, of about 50% at 0.5 μM, of about 82% at 1μM and achieved over 95% at 2.5 μM (Fig. 2). Conversely, HYB 508 caused a growth inhibition which at the highest does of 2.5 μM achieved 10%. See Figure 5.

On page 90, please delete the paragraph at lines 6-19 and replace it with the following paragraph:

We investigated the antitumor activity of HYB 165 (AS RIα) in nude mice bearing GEO colon cancer xenografts, using either the intraperitoneal (i.p.) or the oral route of administration. When established GEO tumors of approximately 0.2 cm^3 were detectable, groups of 10 mice were treated i.p. with either HYB 165 or a control modified backbone oligonucleotide with a scrambled sequence, at 5 or 10 mg/kg/dose, daily on days 7 to 11 and 14 to 18. Figure 16A shows that i.p. administration of HYB 165 caused a dose-dependent inhibition of growth up to 40% at a dose of 10 mg/kg/dose. The control oligonucleotide produced no inhibition at 10 mg/kg/dose.

On pages 91-93, please delete the paragraph at lines 27-35, 1-33, and 1-2 and replace it with the following paragraph:

As illustrated in Figure 17A, treatment with either taxol or the HYB 165 alone inhibited tumor growth as compared to control untreated mice or to mice treated with the scramble MBO. HYB 165 was more effective than taxol, causing over 50% inhibition of tumor size at the completion of the three cycles of treatment. However, shortly after the end of treatment, GEO tumors resumed the growth rate of those in untreated mice or in mice treated with the scramble MBO. When taxol and HYB 165 were used in combination, a marked and sustained inhibition of tumor growth was observed. In fact, tumors of mice treated with taxol and HYB 165 grew very slowly for approximately 60 days following the end of treatment, at which time they

resumed a faster growth rate (Figure 17A). Administration of the scramble MBO in combination with taxol produced an effect similar to that of taxol alone. Within approximately 5 weeks, GEO tumors reached a size not compatible with normal life in all untreated mice and in mice treated with the scramble MBO (Figure 17B). A slight increase in survival time was observed in the group treated with taxol alone, an effect similar to that observed in mice treated with taxol followed by the scramble MBO (data not shown). Treatment with HYB 165 alone also increased survival time as compared to the control group. The delayed GEO tumor growth observed in the group treated with taxol in combination with HYB 165 was accompanied by a prolonged mouse life span, when analyzed with the log-rank test (N. Mantel, *Cancer Chem. Rep.*, 163-170 (1966)), was significantly different as compared to controls ($P < 0.0001$), to the taxol-treated group ($P < 0.0001$) or to the group treated with scramble MBO plus taxol ($P < 0.0001$). In fact, the only mice still alive at 10 weeks after tumor cell injection were those treated with the combination of taxol and HYB 165. Furthermore, about 50% of the mice in this group were still alive after 15 weeks. The combined treatment with taxol and HYB 165 was well tolerated, since no weight loss or other signs of acute or delayed toxicity were observed.

On page 93, please delete the paragraph at lines 6-8 and replace it with the following paragraph:

Cooperative antitumor effect of HYB 165 with taxol is accompanied by inhibition of new vessel formation and growth factor production.

On page 93, please delete the paragraph at lines 10-22 and replace it with the following paragraph:

Tumor specimens from the different groups of mice were examined by histochemical analysis at different time points to evaluate the expression of a variety of biological parameters. Results of the analysis performed on tumor specimens after two cycles of treatment are presented in Figure 18. Treatment with HYB 165 inhited expression of the target RIα protein in the tumor. This effect was further increased when HYB 165 was used in combination with taxol. No other treatment was able to affect RIα expression. These results suggest that inhibition of RIα expression is not dependent on growth inhibition.

On pages 94-95, please delete the paragraph at lines 12-34 and 1-2 and replace it with the following paragraph:

In recent years, the critical role of tumor-induced neovascularization in neoplastic development, progression and metastasis has been elucidated (J. I. Fokman, In: J. Mendelsohn *et al.*, eds., *The Molecular Basis of Cancer*, pp 206-232, Phaladelphia: WB Saunders (1995). A reliable histologic estimate of novel blood vessels on tumor specimens is the microvessel count (MVC) in the most intense areas of neovascularization. In the present study, tumor-induced neovascularization was quantified by immunohistochemistry using an anti-Factor VIII related antigen monoclonal antibody (N. Weidner, *Breast Cancer Res. Treat.*, 36:169-180 (1995)). As shown in Figure 18, a significant inhibition of staining was obtained with HYB 165 (about 80%) as well as with taxol (over 60%), as compared to samples from untreated mice or mice treated with the scramble MBO. Combined treatment with taxol and HYB 165 completely suppressed vessel formation in GEO tumors, demonstrating that the cooperative antitumor effect was associated with the marked inhibition of several factors controlling cell cycle, proliferation and angiogenesis of this human colon cancer model.